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AN INDIGENEOUS METHOD TO ESTABLISH DETECTION LIMIT AND QUANTIFICATION LIMIT FOR FOUR BASIC BIOCHEMICAL PARAMETERS

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ABSTRACT
Laboratory diagnosis is an integral part of healthcare delivery systems. There has been rapid improvement in laboratory diagnosis due to the availability of a wide range of sophisticated state of art fully automated discrete selective analysers to use. All clinical laboratories are now employing reagent kits supplied by reputed companies for the measurements of almost all analytes. Accreditation by NABL has given an awareness to improve standardized and reliable laboratory reports for the correct diagnosis. However, only very few laboratories check certain indicators mentioned in the kit leaflets, among which DL and QL are the most important. NABL too has stressed the importance of checking the detection limit and linearity atleast for some analytes, if not for all. This research paper presents the DL and QL check done for four analytes viz Glucose, Urea, Creatinine and Calcium. The DL obtained for the above analytes are better than those mentioned in the kit leaflets. Glucose 1.0 mg/dL, Urea 0.73 mg/dL, Creatinine 0.04 mg/dL and Calcium 0.53 mg/dL against Glucose 6.04 mg/dL, Urea 3 mg/dL , Creatinine 0.29 mg/dL and Calcium 0.36 mg/dL given in the kit leaflets. The QL obtained for the above parameters are Glucose 600.8 mg/dL, Urea 300 mg/dL, Creatinine 32.5 mg/dL and Calcium 23.05 mg/dL against Glucose 614 mg/dL, Urea 303 mg/dL, Creatinine 32.5 mg/dL and Calcum 17.24 mg/dL provided in the kit leaflets. This study will be a guide for other laboratories to do such experiments to establish their own DL and QL and to fulfill NABL criteria.

KEYWORDS: DL, QL, CLIA, Validation, Glucose, Urea, Creatinine, Calcium.

INTRODUCTION
In any quantitative biochemical analysis, there are two important factors involved viz Detection Limit (DL) and Quantification Limit (QL). Generally most Indian laboratories employ kits supplied by commercial organization to measure the anlaytes. Many laboratory accreditation bodies stress the importance of establishing the laboratory's own DL & QL, however only very few laboratories follow this guideline. This research work is an attempt to establish DL & QL for few basic biochemical analytes and to compare the results with those found in the kit leaflets.

The quality of an analytical method developed is always appraised in terms of suitability for its intended purpose, recovery, requirement for standardization, sensitivity, analyte stability, ease of analysis, skill subset required, time and cost in that order. It is highly imperative to establish through a systematic process that the analytical method under question is acceptable for its intended purpose. DL and QL are two important performance characteristics in method validation. DL and QL are terms used to describe the smallest as well as highest concentration of an analyte that can be reliably measured by an analytical procedure. There has often been a lack of agreement within the clinical laboratory field as to the terminology best suited to describe this parameter. Likewise, there have been various methods for estimating it.[1] Both Clinical Laboratory Improvement Amendments of 1988 (CLIA) and the College of American Pathologists (CAP) Laboratory Accreditation Program require clinical laboratories to verify performance characteristics of quantitative test systems. Laboratories must verify performance claims when introducing an unmodified, approved test system, and they must comply with requirements for periodic calibration and calibration verification for existing test systems. They must also periodically verify the

analytical measurement range of many quantitative test systems. Calibration verification and QL and analytical measurement range verification should be performed using suitable materials with assessment of results using well-defined evaluation protocols.[2]

In a study, the inter and intra assay % CV were 1.54 and 1.04 %, the DL 1.0324, Analytical Measurement Range (AMR) 1.26 and the assay was linear with different dilutions. Lean concept was verified with high recovery %. Validation has ensured the accurate and precise results in a clinically relevant turnaround time.[3] The present overview of validation and verification procedures in clinical chemistry focuses on the use of harmonized concepts and nomenclature, fitness-for-purpose evaluations and procedures for minimizing overall measurement and diagnostic uncertainty. The need for mutually accepted validation procedures in all fields of bioanalysis becomes obvious when they implement international accreditation and certification standards or their equivalents. The guide on bioanalytical method validation published by the US FDA in 2001 represents a sensible compromise between thoroughness and cost–effectiveness. Lacking comprehensive international agreements in the field, this document has also been successfully adapted in other fields of bioanalysis. European and international efforts aiming for consensus in the entire field of bioanalysis are currently being made. Manufacturers of highly automated in vitro diagnostic methods provide the majority of measurement methods used in unmodified in clinical chemistry. Validated by the manufacturers for their intended use and fitness-for-purpose, they need to be verified in the circumstances of the end-users. As yet, there is unfortunately no general agreement on the extent of the verification procedures needed.[4]

Quantitation of the total protein content in a sample is a critical step in protein analysis. Molecular UV-VIS absorption spectroscopy is very efficient in quantitative analysis such as protein quantitation and has extensive applications in chemical and clinical laboratories worldwide. Among the various techniques for protein assay, biuret test is of particular interest and in a study to verify the sensitivity of biuret assay to protein samples with concentrations ranging from 0.0100 to 5.00 mg/mL, albumin chicken egg was used as the protein sample. The assay was assessed to determine the range of concentration in which it will show QL. The biuret test exhibited QL in a wide range of concentrations (0.1000 ± 0.0004 to 5.00 ± 0.02 mg/mL). The starting protein concentration range established for the calibration curve of this assay is lower than the literature value of 1 mg/mL, providing more workable range for biuret test.[5] Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and Quality Assurance Programs. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishing product-specific acceptance criteria and stability of results.Validation should demonstrate that the analytical procedure is suitable for its intended purpose. Design of experiment is a powerful tool for the method characterization and validation. Analytical professionals should be comfortable to use it to characterize and optimize the analytical method. An effective analytical method development and its validation can provide significant improvements in precision and a reduction in bias errors. It can further help to avoid costly and time consuming exercises.[6]

Acceptable calibration verification evaluation is significantly related to acceptable rates for most analytes, including albumin, calcium, chloride, glucose, iron, magnesium, sodium, total bilirubin, uric acid, high-density lipoprotein cholesterol, triglycerides, alkaline phosphatase, alanine and aspartate aminotransferases, digoxin, gentamicin, phenobarbital, procainamide, and thyroxine. There is a consistent and strong relationship between calibration verification problems in the Linearity Surveys (LS) and failure rates in the CAP chemistry surveys. Laboratories with poor calibration evaluations on LS have higher unacceptable rates on proficiency tests. Individual laboratories who were rated linear and whose calibration was verified by LS have lower unacceptable rates.[7] In Linear relationships between response and concentration were used to estimate the DL and QL for five avermectins: emamectin, abamectin, doramectin, moxidectin, and ivermectin. Estimation of DL and QL was based on the SD of residual and *y*-intercept of the regression line at low concentrations of avermectins, using the dispersive solid-phase extraction procedure. Avermectin extracts were analyzed using liquid chromatography tandem mass spectrometry. Based on the regression slope, DL and QL were higher at concentrations of 0.3–0.4 µg/kg and 1 µg/kg, respectively, for all avermectin compounds. QL was performed by linear regression, which incorporated a regression model, outlier rejection, and evaluation of the assumption with a significant test. For all avermectins, there is a significant correlation between response and concentration in the range 1–15 µg/kg, and the *y*-intercept passes through origin (zero).[8]

MATERIALS AND METHODS

Daily leftover patients serum samples were pooled into a plastic container until about 50 ml of serum was obtained. This pooled serum was tested for HBsAg,and HIV antibodies and were found to be negative. The pooled sera were then left in the freezer (< 0°C) overnight. Next day morning, the container was removed, kept upside down on a 100mL measuring cylinder and collected the first 30mL, which is said to be the more concentrated. The sera thus collected was then analysed and the values obtained for the parameters studied in this project were given in Table 1. Appropriate addition of the analytes was done using AR chemicals so as to get a higher level close to the upper limit mentioned in the kit leaflet for each analyte.

After mixing well, the concentration of each analyte was then checked to ensure that added analytes has made the level close to the upper limit of QL mentioned in the kit leaflet.

A serial dilution was done so as to get 10 different dilutions to get a wide range of concentrations, the lowest levels being close to the DL mentioned in the kit leaflet for each analyte. The diluted samples were analysed and the absorbance readings were calculated using calibrator value and its absorbance.

Randox RX Imola analyser and the kits supplied by the company for each analyte was used. Biorad level 1 & 2 accuracy controls were used to validate the reliability of results obtained in this study.

RESULTS

The preliminary levels of the pooled serum for each analyte, the amount to be spiked by adding the required amount of each analyte, the theoretical and observed values are given in Table 1.

Table 1

S.No.	Analyte Name	Initial Level (mg/dL)	Level to be Increased (mg/dL)	Amount to be added to 50 mL of Pooled serum	Theoretical value (mg/dL)	Obtained value (mg/dL)
1	Glucose	80	650	0.32 gm of Dextrose	650	600.8
2	Urea	25	350	0.18 gm of Urea	350	300.0
3	Creatinine	0.9	35	0.02 gm of Creatinine	35	32.5
4	Calcium	9.2	25	0.04 gm of Calcium carbonate	25	23.05

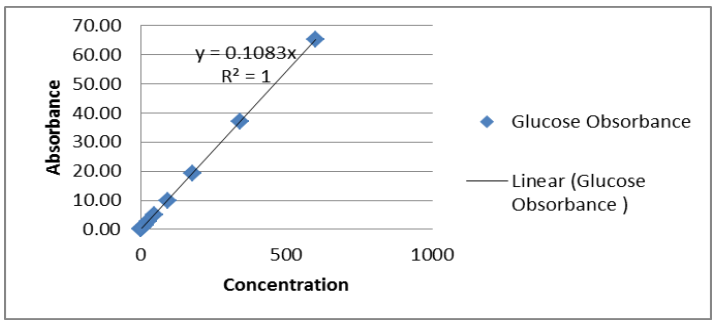
The results obtained using the serially diluted samples for the four analytes studied are presented in Table2. This table also gives the DL and QL obtained for the 4 analytes together with the values provided in the kit leaflet.

Table 2

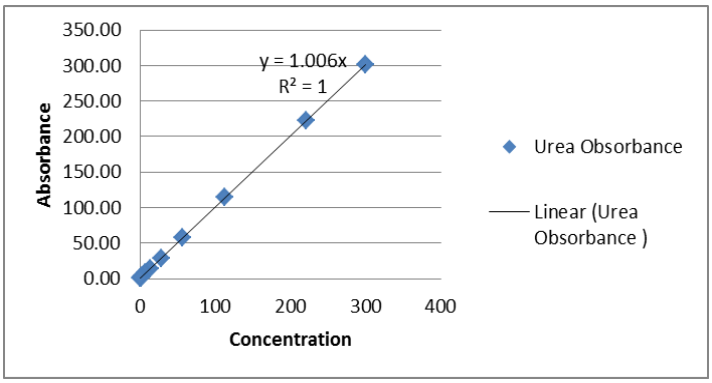
1:1 Serial No. of Dilution	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Calcium (mg/dL)
Direct	600.8	300.0	32.5	23.05
Dilution 1	340.9	220.86	20.65	10.3
Dilution 2	176.9	113.16	11.19	5.4
Dilution 3	91.8	57.17	5.76	4.1
Dilution 4	47.4	28.82	2.99	1.43
Dilution 5	24.1	14.45	1.46	1.88
Dilution 6	12.6	7.87	0.74	1.26
Dilution 7	6.4	3.72	0.37	1.11
Dilution 8	3.2	2.23	0.17	0.61
Dilution 9	1.7	1.31	0.07	0.73
Dilution 10	1.0	0.73	0.04	0.53
Upper limit obtained	600.8	300	32.50	23.05
Upper limit Provided in kit leaflet	614	303	32.5	17.24

The results obtained as shown in Table 2 were used to plot graphs (Concentration vs Absorbance) passing through the origin, extending up to the upper QL obtained in this study. These 4 graphs illustrate the DL obtained along with the QL for the 4 analytes studied together with R^2.

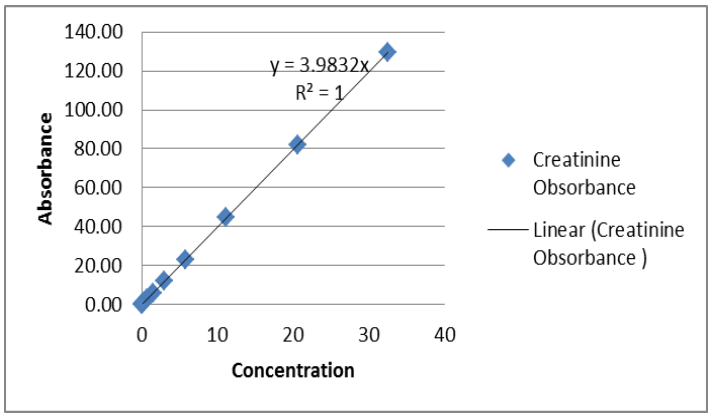
GLUCOSE



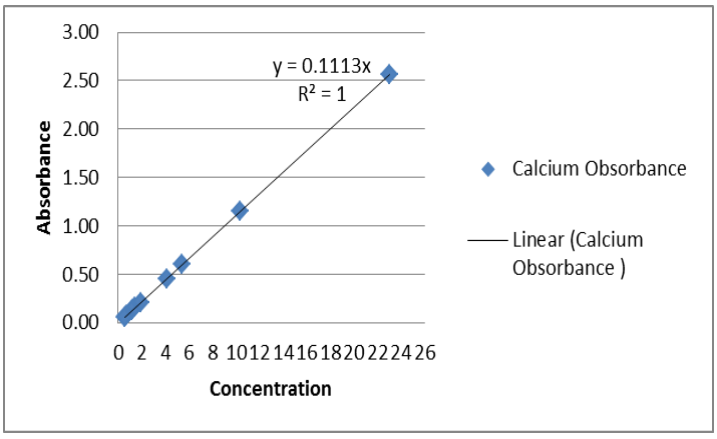
UREA



CREATININE



CALCIUM



DISCUSSION

Many studies have been done in the past to establish DL and QL for biochemical parameters. Both CLIA and CAP have stressed the importance of establishing such indicators.[1, 2] Only very few clinical laboratories comply with such recommendations. In the past DL and QL have been done for protein including AMR.[5] However there are lacunae in such studies in Indian laboratories and available data in such studies are very few. This study has established DL and QL for the most 4 basic parameters viz glucose, urea, creatinine and calcium and compared the values obtained with those found in the kit leaflets and in a study, the results obtained for total protein is in agreement with this study [5] as well as some studies based on proficiency surveys.[7] In some extraction techniques, both DL and QL were higher and this study has established lower figures for both DL and QL for 4 parameters.[8] The outcome of this study is better than previous studies and will serve as guidelines in establishing DL and QL for biochemical analytes.

CONCLUSION

The outcome of the study has established an indigenous method which is simple and easy to use procedure to establish DL and QL in each laboratory. The method presented is simple to use and could be done by any laboratory technician if proper instructions are given. The raw material used in this experiment is from leftover, non infectious and HBsAg & HIV antibodies negative samples from normal patients. The results

obtained for DL and QL are found to be better than those provided in the kit leaflet. The DL and QL obtained will help the laboratory to measure very low concentrations in paediatric samples and also save on dilution and repeat analysis as the result obtained up to the linear range could be used straight way. Further studies are required to establish DL and QL for other analytes such as cholesterol and other important enzymes.

CONFLICT OF INTEREST: None.

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